Determination of Copper and Zinc in Various Beans Samples Followed by Flame Atomic Absorption Spectrometry: A Multivariate Strategy

[1] Nazia Fareed, [1] Iffat Abdul Tawab Khan, *[2] Ghulam Fareed
[1]*Department of Chemistry, Federal Urdu University of Arts, Science & Technology, Gulshan-e-Iqbal, Karachi-75260, Pakistan.*
[2]*Pharmaceutical Research Center, PCSIR Laboratories Complex, Karachi-75280, Pakistan.*
fareedchm@yahoo.com*

(Received on 1st June 2016, accepted in revised form 21st September 2016)

Summary: In the present study, flame atomic absorption spectrometry (FAAS) was used to analyze metal contents (Cu(II) and Zn(II)) in a variety of beans samples. Optimized dry wet acid digestion (DWA) technique was employed to digest all the beans samples. These beans samples were collected during 2014-2015 from the local market of Karachi, Pakistan. Plackett-Burman experimental design (PBD) and 2^3+star central composite designs (CCD) were used to optimize the DWA method. Parameter influencing such as sample weight (S. wt), acid volume (AV), temperature of the furnace (T) and intensification time in furnace (t) were explored. From these studies, certain variables proved as significant, and were further optimized by a CCD design, which involve 16 experiments. The results obtained by proposed methodology showed sufficiently high % recoveries 98.6 % for Cu(II) and 97.3 % for Zn(II). Under optimized conditions, the detection limits (3σ) were evaluated to be 11.2 µg/Kg for Cu(II) and 5.09 µg/Kg for Zn(II).

Key words: Multivariate study, Cu (II), Zn (II), Bean, Atomic absorption.

Introduction

Beans are widely used as for food material due to its high nutritive value. Apart from high contents of carbohydrates, proteins and water soluble vitamins, beans are an important source of essential metal ions. The role of these metal ions on human health is undisputable [1]. Zinc and copper present in beans are two of the most metabolically active and essential nutrients for humans which are intensively investigated. However, both can be toxic depending on their concentration [2, 3]. The established biochemically stimulating role of zinc is an fundamental constituent of various metallo enzymes systems which plays catalytic structural and a central role in metabolism of nucleic acid [4]. In addition, zinc also showed to have a significant task in membrane stabilizer, biomembrane formation and function. Zinc also play a significant job in a variety of cell processes together with DNA synthesis normal growth, fetal development, brain maturity, behavioral response, and bone constitution [5, 6]. Deficiency of zinc leads to many clinical manifestations including impairment of the immune system, lungs or intestinal tract manifestation [7, 8]. Cu(II) is also a key constituent of various enzymes systems including super oxide, dismutase (SOD), cytochrome c-oxidase, lysyl oxidase and ceruloplasmin [9-12]. Copper plays a significant task in common neurological conditions including Alzhaimer's disease, the Prion disease and Wilsons's diseases [13]. Toxicity of copper may cause anemia, break of nucleic acid strands as well as destruction of membrane and mitochondria [13, 14].

The digested food samples for the determination of metals have conventionally been analyzed by flame atomic absorption spectrometry [15, 16]. In recent trends multivariate technique is widely used for the optimization of methodology because this technique is faster, more economical, effective [17-19]. Various mean of optimization are available for the extraction of metals in various matrixes, however various techniques are time consuming; for instance the study of multivariable is not allowed. It is therefore, multivariate technique has been encouraged for the optimization of variables [20-23]. Among various designs such as Plackett Burmann design (PBD) [24] and CCD [18] was used for the screening of variables.

In this article, we have described the effect of variables on the extraction of studies metal ions from the beans samples. Various samples of beans were analyzed for the metal contents (Cu(II) and Zn(II)) using flam atomic absorption spectrometer. Several variables (S. Wt, AV, T and t) were investigated against the recovery of metals. Among these variables some are regarded as significant and few are regarded as less significant which were evaluated by PBD. Further optimization was done by CCD design in order to determine the most momentous variables.

*To whom all correspondence should be addressed.

Experimental

Reagents and Glassware

All chemicals were utilized of analytical grade Merck, (Darmstadt, Germany). De-ionized water was used throughout the studies. Concentrated (65%) nitric acid was used of analytical grade Merck (Darmstadt, Germany). Copper and zinc standard solutions were prepared from their stock solutions, (1000 μgmL^{-1}) Fluka Kamica (Switzerland) of subsequent metal ions. Glassware were kept overnight in 10% (v/v HNO_3) and rinsed with deionized water and dried in an oven before use.

Instrumentation

A Perkin Elmer Model 3300 atomic absorption spectrophotometer was used to analyze zinc and copper in beans samples. The concentrations of studied metals were determined from calibration graph after adjustment of the absorbance for the signal from the suitable reagent blank.

Sampling

Ten different verities of beans were collected from various local markets of Karachi, Pakistan. The samples were collected in the year of 2014-2015. The samples were dried at room temperature and stored on various dates to monitor the deviation of metallic interferences due to environmental conditions. Dried sample were grounded with mortal and piston in smaller thick pieces and then digested according to AOAC modified method of 944.02 with nitric acid and then thereafter samples were analyzed by FAAS.

Conventional Digestion Method

Collection, pretreatment and digestion of samples are the most important steps of any kind of chemical analysis. Ten different beans samples according to their verities were collected from local market of Karachi city. All collected samples of beans were properly cleaned. Moisture was removed at 110-120°C for 6-7 hours by using oven. Accurately about 2 g of dried sample was weighed by digital weighing balance. Digestion is followed by AOAC, in which dry wet ashing method was applied. First of all the sample was charred on blue flam till complete removal of hydrocarbons. Then sample was oxidized using 2 mL concentrated HNO_3 and heated to 2 minutes, on heating acid was evaporated and sample was ready to ignition. Oxidize sample was then placed in furnace at 500°C and after complete ignition grey or white ash was obtained which were actually the metal oxides. 5 mL concentrated HCl was added in the sample and then heated at low flam till complete dryness. 2mL more concentrated HCl at room temperature and heated to hotness. Then it was transferred to 100mL volumetric flask and made it up to 100.0 mL with distilled deionized water. Insoluble particles were removed by filter paper and filter paper was washed by distilled deionized water to ensure that all metal chlorides were transferred to the flask. The blank was carried out in the same way using the solvent alone.

Statistical Evaluation

Statistical Analysis was carried out using Minitab 16 and Statistica 10. In case of PBD four variables at two levels, twelve experiments are illustrated instead of the $2^7 = 128$ mandatory for complete factorial design.

Calibration and Accuracy

To ensure the accuracy of the experiment kidney bean was selected to detect the precision and % recovery with five similar samples (n=5). Calibration, Standard addition graphs and % RSD were determined for Cu(II) and Zn(II), which proved the method used was accurate and precise Table-4. The limit of detection and limit of quantification are represented by

$$\text{LOD} = 3^{x}\frac{s}{m} \text{ and LOQ} = 10^{x}\frac{s}{m}$$

Respectively, where *s* is the standard deviation of ten analysis of reagent blank and *m* is the slope of calibration or standard addition graph was acquired for copper and zinc. The limit of detection (LOD) for the two studied metals, 11.2 µg/Kg for Cu(II) and 5.09 µg/Kg for Zn(II) and limit of quantification (LOQ) for Cu(II) 38.2 µg/Kg and for Zn(II) 15.7µg/Kg were calculated.

Results and Discussion

Optimization Strategy by Multivariate Strategy

The multivariate strategy was performed in two steps *i.e*. PBD and CCD designs. Four different variables sample weight (S. wt), acid volume (AV), temperature of the furnace (T) and time in furnace (t) were investigated by PBD for the recovery of the metals. The values of each variable were represented in two levels (+) maximum and (-) minimum as illustrated in Table-1.

Table-1: Variables and their levels used for multivariate (PBD and CCD) designs in the factorial design.

Variables	Symbol	Low level (-)	High level (+)
Sample Weight (g)	S. Wt	2	5
Acid Volume (HNO_3) (mL)	AV	0.5	2
Temperature of furnace (°C)	T	400	500
Intensification time in Furnace (h)	t	2	3

Plackett-Burman Design (PBD)

The PBD was employed as a screening approach intends to establish the important variables that influence on metal recovery and choosing best appropriate digestion parameters. The purpose of PBD design minimizes the time of method and provides fewer ambiguous digestion parameters, hence assisting the data elucidation.

Twelve experiments were performed for implementation of the design matrix; the resulting values (1-12) are being the % recovery of Cu(II) and Zn(II) average value of triplicates. In addition the evaluation of interface among variables was studied, while the consequences of some variable are considered as momentous and few of them were less significant (Table-2).

Table-2: Plackett-Burman Design.

Run	S.Wt	AV	T	t	% Recovery (mean±S.D.) Cu(II)	Zn(II)
1	+	-	+	-	60.12 ±3.41	52.30 ± 2.23
2	+	+	-	+	79.45 ± 3.98	71.65 ±2.54
3	-	+	+	-	87.42 ± 5.50	79.68 ± 3.98
4	+	-	+	+	62.31 ± 2.93	54.63 ± 4.43
5	+	+	-	+	82.32 ± 2.87	74.71 ± 2.98
6	+	+	+	-	92.31 ± 4.01	94.69 ± 3.07
7	-	+	+	+	98.61 ± 5.54	97.35 ± 5.09
8	-	-	+	+	66.39 ± 4.98	58.13 ± 2.87
9	-	-	-	+	66.86 ± 5.32	61.28 ± 3.78
10	+	-	-	-	37.62 ± 1.23	32.35 ± 2.09
11	-	+	-	-	76.10 ± 1.87	75.72 ± 3.32
12	-	-	-	-	36.21 ± 6.32	31.13 ±2.11

2^3+star Central Composite Designs

In order to acquire the best possible values of each factor involved in specific system the central composite designs (CCDs) are the most extensively employed. A CCD was carried on the basis of the results of pareto charts. The most significant three variables were selected and then optimized by CCD design and were optimized involving 16 experiments to afford the maximum metal recovery. The statistically three most significant variables *i.e*., acid volume (AV), temperature of the furnace (T) and time of furnace (t) were evaluated for the determination of Cu (II) and Zn (II) in beans samples as shown in Table-3.

On the basis of CCD following optimal extraction conditions for DWA were selected (AV) = 2 mL, (T) = 500 °C and (t) = 3 h which have significant effect on extraction efficiency of studied metals and their optimum responses were studied by response surface methodology (RSM) (Fig. 3-6).

Table-3: Central Composite Design for the % recovery of Cu(II) and Zn(II) vs. AV, T and t.

Run	AV	T	t	% Recovery (mean±S.D.) Cu(II)	Zn(II)
1	-	-	-	45.21 ± 2.53	46.42 ± 2.32
2	+	-	-	72.33 ± 1.98	73.26 ± 2.11
3	-	+	-	52.58 ± 5.34	56.44 ± 1.92
4	+	+	-	91.37 ± 1.34	94.50 ± 2.10
5	-	-	+	52.16 ± 2.76	54.35 ± 3.08
6	+	-	+	88.23 ± 2.86	90.11 ± 4.87
7	-	+	+	60.22 ± 3.68	59.57 ± 3.21
8	+	+	+	98.78 ± 6.87	99.69 ± 1.87
9	$-a^a$	$^2k_0$	$^3k_0$	47.20 ± 4.98	44.23 ± 5.32
10	$+a^b$	$^2k_0$	$^3k_0$	39.25 ± 1.32	37.18 ± 4.34
11	$^1k_0$	$+a^c$	$^3k_0$	48.59 ± 1.54	49.35 ± 2.22
12	$^1k_0$	$+a^d$	$^3k_0$	52.32 ± 2.23	50.27 ± 1.12
13	$^1k_0$	$^2k_0$	$+a^e$	51.17 ± 5.35	49.22 ± 2.94
14	$^1k_0$	$^2k_0$	$+a^f$	67.23 ± 4.87	65.59 ± 3.65
15	$^1k_0$	$^2k_0$	$^3k_0$	70.52 ± 1.33	68.72 ±2.04
16	$^1k_0$	$^2k_0$	$^3k_0$	70.79 ± 2.48	69.07 ± 2.72

Factors: 3, replicates: 1, design: 8, run: 16, center point total: 23.
$^1k_0$= 1.250mL, $^2k_0$= 450°C, $^3k_0$= 2.50h, $-a^a$= -0.011mL, $+a^b$= 2.51134mL, $+a^c$= 365.910°C, $+a^d$= 534.090°C, $+a^e$= 1.65910h, $+a^f$= 3.34090 h.

Table-4: The results of standard addition / recovery for Cu (II) and Zn (II) in bean sample (Kidney bean), n=5.

Added value (µg/ml)	Found value	%RSD	% Recovery
Copper (Cu)			
0.0	19.81	1.37	98.82
5	24.70	2.01	98.58
10	29.91	1.87	98.93
15	34.78	1.48	99.17
Zinc (Zn)			
0.0	89.2	1.78	99.10
5	94.13	2.91	97.78
10	99.01	1.23	98.77
15	105.1	1.91	99.37

Effects of Variables

Four variables including S.Wt, AV, T and t were optimized by PBD. However, on the basis of results (Table-2) and pareto charts some factors were shown to insignificant and while few of them were found to be significant such as AV, T and t. Hence, when all the factors were at most favorable level the maximum % recoveries were observed for both metals 98.6 % for Cu(II) and 97.3 % for Zn(II) as shown in experiment no.7.

From the results of PBDs and CCD, it was undoubtedly examined that a significantly high extraction efficiency was obtained for Cu(II) and Zn(II) at high level of AV,T. The experimental results showed that AV was found to be very important and key factor for optimization of experimental conditions which influence extraction efficiency of studied metal ions.

The results showed that % recovery of studied metals increased with increase in AV and T

whereas the rest of the variables were found at low (-) level but the % recoveries for Cu(II) and Zn(II) 87.4 % and 79.6 % were obtained respectively (experiment No.3). However, when AV is low (-) and the remaining variables were found at high (+) level but the minimum recoveries *i.e*. 62.3 % for Cu(II) and 54.6 % for Zn(II) were obtained (experiment No. 4). The correlation and interactions between the variables can be examined by pareto chart as shown in Fig. 1 and 2.

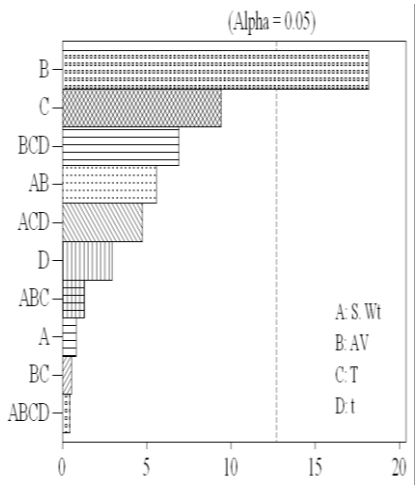

Fig. 1: Pareto chart for Cu (II).

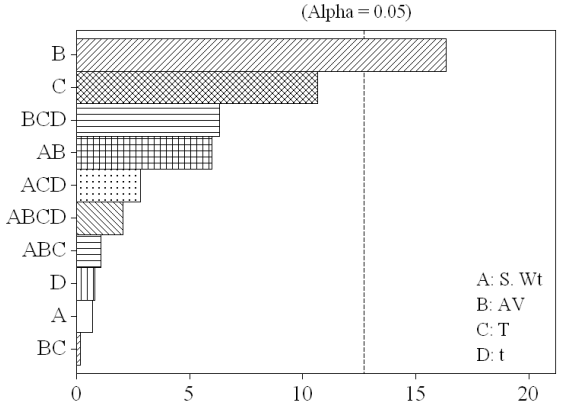

Fig. 2: Pareto chart for Zn (II).

For sequential experimentation CCD was used for investigating reasonable and significant factors. Thus, the three most important factors including AV, T and t were studied and optimized by CCD involving 16 experiments. The results showed that at high (+) level AV and T and low (-) level t the % recoveries were 91.3 % for Cu(II) and 94.5 % for Zn(II) (experiment No. 7). However, when the concentrations of AV, T and t were at maximum level (experiment No. 8) the maximum % recoveries for both metal ions 98.7 %for Cu(II) and 99.6 % for Zn(II) were obtained, indicating the high (+) t increased the extraction efficiency of studied metal ions.

The investigation of estimated three dimension (3D) surface response for factors AV, T and AV, t was studied by response surface methodology and can be visualized by 3D graphs. From the three factors only one factor was fixed at central points while the remaining factors were at variable conditions. The plot depicted for each pair of variables Vs % recovery of Zn(II) and Cu(II) as shown in Fig. 3-6.

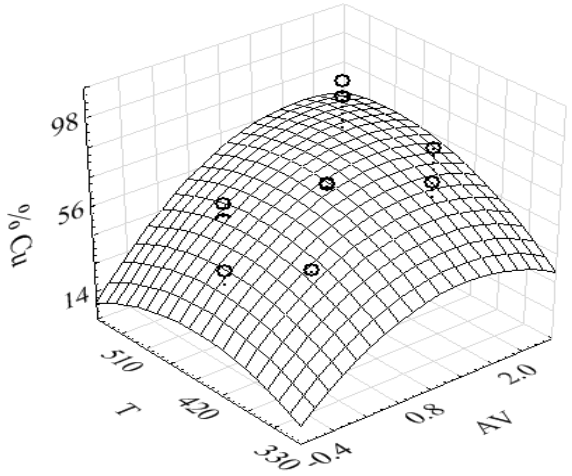

Fig. 3: 3D Central Composite Design for the % recovery of Cu (II) vs. AV and T.

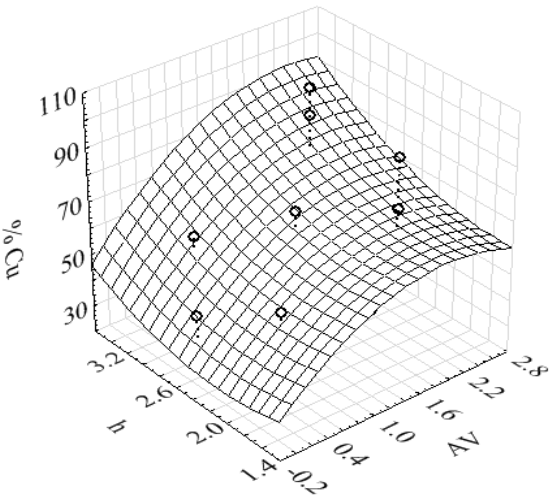

Fig. 4: 3D Central Composite Design for the % recovery of Cu (II) vs. AV and h.

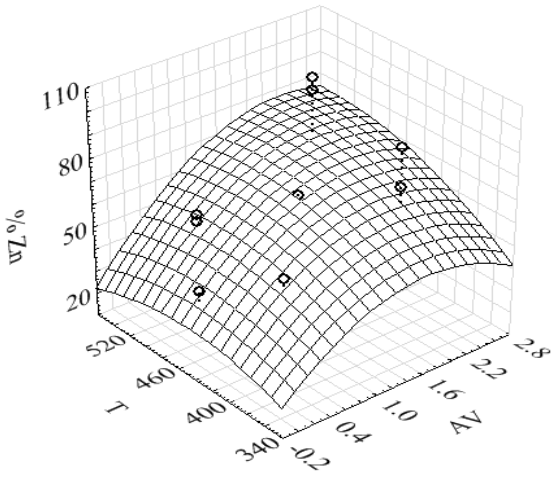

Fig. 5: 3D Central Composite Design for the % recovery of Zn (II) vs. AV and T**.**

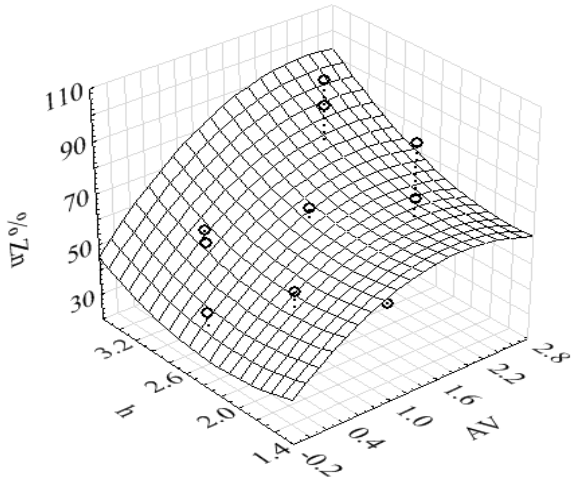

Fig. 6: 3D Central Composite Design for the % recovery of Zn (II) vs. AV and h.

Evaluation of Copper and Zinc in Beans samples

Analytical results of various beans samples obtained by proposed method are depicted in Table-5. Copper and zinc can be taken into body from food, which are good for health and the important basic minerals to prevent and control various diseases. The results indicated that the ten analyzed beans samples were abundant in copper and zinc elements and they were of interest due to their various health benefits as already described above in detail. The results also showed that copper and zinc contents were high in soya bean as compared to the other tested samples.

Table-5: Determination of Cu (II) and Zn (II) in beans samples (µg/g).

S. No	Name of Sample	Copper	Zinc
1	French Beans	11.31	39.31
2	Kidney Beans	19.83	89.41
3	Broad Beans	15.21	32.39
4	Cluster Beans	14.63	54.65
5	Hyacinth Bean	12.81	62.89
6	Black Eyed Beans	16.34	32.76
7	Soya Beans	39.61	98.70
8	Mung Beans	13.39	25.21
9	Pinto Beans	27.06	36.81
10	White Beans	14.32	38.41

Conclusion

The optimized method used for the determination of Cu (II) and Zn (II) in varieties of beans samples is precise and simple. Moreover, from nutritional point of view soya beans and kidney beans are rich of copper and zinc.

References

1. W. Kmiecik, Z. Lisiewska and G. Jaworska, Content of Ash Components in the Fresh and Preserved Broad Bean (Vicia Faba V Major), *Journal of Food Composition and Analysis*, **13**, 905 (2000).
2. N. Jalbani, F. Ahmed, T. G. kazi, U. Rashid, A. B. Munshi and A. Kandhro, Determination of Essential Elements (Cu, Fe and Zn) in Juices of Commercially Available in Pakistan, *Food Chem. Toxicol.*, **48**, 2737 (2010).
3. P. C. Onianwa, I. G. Adetola, C. M. A. Iwegbue, M. F. Ojo and O. O. Tella, Trace Heavy Metals Composition of Some Nigerian Beverages and Food Drinks, *Food Chem.*, **66**, 275 (1999).
4. A. Maiga, D. Diallo, R. Bye and B. S. Paulsen, Determination of Some Toxic and Essential Metal Ions in Medicinal and Edible Plants from Mali, *J. Agric. Food Chem.*, **53**, 2316 (2005).
5. F. Piao, K. Yokoyama, N. Ma and T. Yamauchi, Subacute Toxic Effects of Zinc on Various Tissues and Organs of Rats, *Toxicology Letters*, **145**, 28 (2003).
6. M. Hambidge, Human Zinc Deficiency, *The Journal of Nutrition*, **130**, S1344 (2000).
7. A. S. Prasad, Discovery of Human Zinc Deficiency and Studies in an Experimental Human Model, *Am. J. Clin. Nutr.*, **53**, 403 (1991).
8. A. H. Shankar and A. S. Prasad, Zinc and Immune Function: The Biological Basis of Altered Resistance to Infection, *Am. J. Clin. Nutr.*, **68**, 447S (1998).

9. J. F. B. Mercer, The Molecular Basis of Copper-Transport Diseases, *Trends Mol. Med.*, **7**, 64 (2001).
10. G. G. Zucconi, S. Cipriani, R. Scattoni, I. Balgkouranidou, D. P. Hawkins and K. V. Ragnarsdottir, Copper Deficiency Elicits Glial and Neuronal Response Typical of Neurodegenerative Disorders, *Neuropathology and Applied Neurobiology*, **33**, 212 (2007).
11. A. Rinaldi, Meeting Report – Copper Research at the Top, *BioMetals*, **13**, 9 (2000).
12. S. G. Kaler, Metabolic and Molecular Bases of Menkes Disease and Occipital Horn Syndrome, *Pediatr. Dev. Pathol.*, **1**, 85 (1998).
13. V. Desai and S. G. Kaler, Role of Copper in Human Neurological Disorders, *Am. J. Clin. Nutr.*, **88**, 855S (2008).
14. A. B. Chukhlovin, S. V. Tokalov, A. S. Yagunov, J. Westendorf, H. Reincke and L. Karbe, In Vitro Suppression of Thymocyte Apoptosis by Metal-Rich Complex Environmental Mixtures: Potential Role of Zinc and Cadmium Excess, *Sci. Total Environ.*, **281**, 153 (2001).
15. M. C. Yebra-Biurrun, S. Cancela-Pérez and A. Moreno-Cid-Barinaga, Coupling Continuous Ultrasound-Assisted Extraction, Preconcentration and Flame Atomic Absorption Spectrometric Detection for the Determination of Cadmium and Lead in Mussel Samples, *Anal. Chim. Acta*, **533**, 51 (2005).
16. M. Soylak, I. Narin, M. d. A. Bezerra and S. L. C. Ferreira, Factorial Design in the Optimization of Preconcentration Procedure for Lead Determination by Faas, *Talanta*, **65**, 895 (2005).
17. T. G. Kazi, M. B. Arain, M. K. Jamali, N. Jalbani, H. I. Afridi, R. A. Sarfraz, J. A. Baig and A. Q. Shah, Assessment of Water Quality of Polluted Lake Using Multivariate Statistical Techniques: A Case Study, *Ecotoxicol. Environ. Saf.*, **72**, 301 (2009).
18. N. Jalbani and M. Soylak, Ligandless Surfactant Mediated Solid Phase Extraction Combined with Fe3o4 Nano-Particle for the Preconcentration and Determination of Cadmium and Lead in Water and Soil Samples Followed by Flame Atomic Absorption Spectrometry: Multivariate Strategy, *Ecotoxicol. Environ. Saf.*, **102**, 174 (2014).
19. E. Martinez, R. Cela, A. M. Carro, J. C. Cobas and B. Garcia, Chemometrically Guided Sample Composition for Fast Screening of Trace Metals in Water Samples, *J. Anal. At. Spectrom.*, **17**, 1373 (2002).
20. B. J. Ahmed, K. T. Gul, S. A. Qadir, K. G. Abbas, A. H. Imran, A. M. Balal, J. M. Khan and N. Jalbani, Speciation and Evaluation of Arsenic in Surface Water and Groundwater Samples: A Multivariate Case Study, *Ecotoxicol. Environ. Saf.*, **73**, 914 (2010).
21. N. Jalbani, T. G. Kazi, B. M. Arain, M. K. Jamali, H. I. Afridi and R. A. Sarfraz, Application of Factorial Design in Optimization of Ultrasonic-Assisted Extraction of Aluminum in Juices and Soft Drinks, *Talanta*, **70**, 307 (2006).
22. W. N. L. Dos Santos, C. M. C. Santos and S. L. C. Ferreira, Application of Three-Variables Doehlert Matrix for Optimisation of an on-Line Pre-Concentration System for Zinc Determination in Natural Water Samples by Flame Atomic Absorption Spectrometry, *Microchem. J.*, **75**, 211 (2003).
23. S. L. C. Ferreira, M. A. Bezerra, W. N. L. dos Santos and B. B. Neto, Application of Doehlert Designs for Optimisation of an on-Line Preconcentration System for Copper Determination by Flame Atomic Absorption Spectrometry, *Talanta*, **61**, 295 (2003).
24. I. Lavilla, B. Pérez-Cid and C. Bendicho, Optimization of Digestion Methods for Sewage Sludge Using the Plackett-Burman Saturated Design, *Fresenius J. Anal. Chem.*, **361**, 164 (1998).